

Identification of new cytotypes of *Valeriana jatamansi* Jones, 1970 (Valerianaceae) from North-Western Himalayan region of India

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Academic editor: I. Gavrillov-Zimin | Received 1 November 2014 | Accepted 11 April 2015 | Published 7 August 2015

<http://zoobank.org/0EAB84A0-0454-4E9D-A614-966EFC399ABB>

Citation: Rani S, Sharma TR, Kapila R, Chahota RK (2015) Identification of new cytotypes of *Valeriana jatamansi* Jones, 1970 (Valerianaceae) from North-Western Himalayan region of India. Comparative Cytogenetics 9(4): 499–512. doi: 10.3897/CompCytogen.v9i4.8875

Abstract

Valeriana jatamansi, a medicinally important species of the family Valerianaceae, has been cytologically studied in different geographical areas of North-Western Himalayan region of India. The tetraploid cytotype with chromosome numbers $2n=32$ is in conformity with the earlier reports of the species from different parts of the world. An octoploid cytotype ($2n=64$) makes a new addition for the species on a worldwide basis, whereas the diploid cytotype ($2n=16$) is new to India have been reported for the first time in India. These cytotypes ($2n=16, 32, 64$) show significant variations with respect to morphology as well as geographical distribution in the Western Indian Himalayas. Further, anomalous populations have been marked with meiotic abnormalities in the form of cytomixis, chromosomal stickiness, unoriented bivalents, formation of laggards and bridges resulting in abnormal microsporogenesis, and production of heterogeneous-sized fertile pollen grains along with reduced pollen fertility.

Keywords

Valeriana jatamansi, cytotypes, meiotic abnormalities, morphovariants, western Himalayas

Introduction

The genus *Valeriana* Linnaeus, 1753 belongs to the family Valerianaceae which comprises 250 species of perennial herbs (Chen et al. 1999) distributed throughout temperate and cold regions of the Northern hemisphere (Bell 2004). In India, 16 species and two subspecies of the genus have been reported; of these 5 species inhabit the high-altitude ranges of the Kumaon and Garhwal regions of central Himalayas (Prakash 1999).

Valeriana jatamansi Jones (= *Valeriana wallichii* de Candolle, 1830) popularly known as Indian valeriana (English), Mushkibala (Kashmiri/ Hindi), Sugandhwala or Tagar (Sanskrit), grows wild in the temperate Himalayan region between 1000 and 3000 m altitude. The species generally grows on sloppy, moist places, damp woods, ditches and along the streams. The species grows particularly in the understory of *Quercus leucotrichophora* Camus, 1938 – *Pinus roxburghii* Sargent, 1897 mixed forests and on grassy habitats of Himalayas. Its occurrence can be observed in different geographical areas and the species possesses diverse morphological and genetic features, affecting its levels of active ingredients. *V. jatamansi* is a perennial herb with thick root stocks, horizontal thick descending fibers with pubescent stem, radical leaves often 1-3 cm in diameter, deeply cordate and usually acute toothed margins. Flowers white, stamens- 3, ovary -3 celled, stigma shortly 2-3 fid and fruits oblong lanceolate and compressed crowned by persistent pappus calyx. The flowering and fruiting time for the species is March–June. The mode of propagation is both sexual through seeds and asexual through rhizome.

The species is being exploited for its roots and rhizomes which contain valepotriates (Chopra 1956) highly effective against leprosy (Kour et al.1999) and curing Lewybody dementia (Bagchi and Hooper 2011). The reported annual collection of *Valeriana jatamansi* from the North-Western Himalayas is about 100 quintals whereas hundreds of quintals *Valeriana* roots are smuggled and go unnoticed. Due to over-exploitation of rhizomes for its medicinal value, it is labeled as endangered plant species in the Himalayan region. In spite of its importance as a medicinal plant, there is a lack of information regarding genetic diversity and chromosome number of different cytotypes present in the area, which is prerequisite for initiation of any systematic breeding programme.

Therefore, keeping in view the economic importance, threatened status of the species, and cytological variability within the species, at present cytomorphological studies have been carried out on a population basis from different areas of the North-Western Himalayas. The present study also discusses the impact of cytomixis on meiotic behavior and reduced pollen fertility and formation of heterogeneous-sized pollen grains in the species.

Material and methods

Material. Material for cytological studies in the form of buds was collected from different parts of the North-Western Himalayas. The propagating material of these plants

was also collected and planted in the experimental fields of Chaudhary Sarwan Kumar Himachal Pradesh Agricultural University, Palampur, India in a Randomized Complete Block design with two replications. The plant to plant distance was kept at 5 cm while row to row distance was kept at 50 cm.

A) Morphological study

Different qualitative morphometric characters were studied for each cytotype to have proper insight on morphological variation present in these cytotypes. For stomatal studies, mature leaves were treated with 10% aqueous solution of potassium hydroxide (KOH) at room temperature for 10–15 min and then epidermal peels so obtained were stained with 10% saffanin in 90% ethanol. In order to reveal the significant difference in the stomata and pollen grain size of diploid, tetraploid and octaploid cytotypes, the t-test was been performed.

B) Cytological studies

For meiotic studies, flower buds were collected from plants growing under natural conditions from selected areas of the Western Himalayas. These flower buds were collected from 15 randomly selected plants of each population and fixed in Carnoy's fixative (6:3:1 ethanol/chloroform/acetic acid v/v/v) for 24 h. Flower buds were washed and preserved in 70% ethanol at 4 °C until used. Smears of appropriate-sized flower buds were made, using the tandard acetocarmine technique (Marks 1954). About 20–50 fresh slides in each case were prepared from different anthers/flowers for different individuals of a particular population and were analyzed in each case. To confirm the chromosome number in case of normal meiosis, around 50 pollen mother cells (PMCs) were observed at different stages of meiosis, preferably at diakinesis/ metaphase-I/ anaphase-I, II. In case of abnormal meiosis, however, more than 300 PMCs were considered to ascertain the type and frequency of various abnormalities per plant. Pollen fertility was estimated by mounting mature pollen grains in glycerol–acetocarmine (1:1) mixture (Belling 1921). Nearly 400–500 pollen grains were analyzed in each case for ascertaining pollen fertility and pollen size. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shrivelled and unstained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Optika Digital Imaging System. The data regarding the number of cytologically worked out species, number of cytotypes, and frequency of polyploids of a particular genus have been compiled on worldwide and India basis from various Chromosomal Atlases and Indexes to Plant Chromosome Numbers by Darlington and Wylie (1955), Fedorov (1974), Moore (1973, 1974, 1977), Kumar and Subramanian (1986), and Khatoon and Ali (1993), various journals, Internet resources, as well as presently studied plants.

Results and discussion

Detailed meiotic studies were carried out on 22 populations of *V. jatamansi* collected from different localities with altitude ranging from 764 to 3647 m above mean sea level in the North-Western Himalayan region of India. The data regarding locality with altitude, latitude and longitude, present meiotic chromosome number, ploidy level and meiotic course of the presently worked out populations have been presented in Table 1.

A) Morphological observations

Morphological variation was assessed on 24 vegetative and reproductive characters of different cytotypes of *V. jatamansi* (Table 2). All the three cytotypes (2x, 4x and 8x) at

Table 1. Information about, locality, latitude and longitude, altitude, meiotic chromosome number, ploidy level and meiotic course of *Valeriana jatamansi*.

S. No.	Locality with latitude and longitude, altitude	Present meiotic chromosome number (2n)	Ploidy level	Meiotic in meters
District Chamba (Himachal Pradesh)				
P-1	Bhali mata/ 32°37'N, 76°0'E/1,900	32	4x	Abnormal
P-2	Salooni/32°43'N,76°03'E/1,900	16	2x	Normal
P-3	Chamba/ 32°33'N, 76°07'E/ 1,200	32	4x	Abnormal
P-4	Leg Valley/31°58'N, 77°06'E/ 1,720	32	4x	Normal
P-5	Tisa/32°32'N, 76°08'E/ 1,220	32	4x	Normal
P-6	Dehgram/32°41'N, 76°08'E/ 2,165	32	4x	Abnormal
District Shimla (Himachal Pradesh)				
P-7	Kandi/32°36'N, 76°02'E/ 854	32	4x	Normal
P-8	Rampur/ 31°58'N,77°06'E/ 1,350	16	2x	Normal
P-9	Pander/ 31°26'N,77°03'E/ 2,236	32	4x	Normal
P-10	Shimla I/ 31°6'N, 77°10'E/ 2,202	32	4x	Abnormal
P-11	Shimla II/31°18'N, 77°20'E/ 1,820	32	4x	Normal
District Mandi (Himachal Pradesh)				
P-12	Rewalsar/31°38'N ,76°50'E/ 1,360	32	4x	Normal
P-13	Rakni/ 31°24'N, 77°07'E/1,649	32	4x	Normal
P-14	Prashar / 31°45'N, 77°6'E / 2,200	32	4x	Abnormal
P-15	Mandi I/ 31°42'N, 76°51'E/ 764	32	4x	Normal
P-16	Mandi II/31°31'N, 76°59'E/ 945	32	4x	Abnormal
District Kullu (Himachal Pradesh)				
P-17	Kullu I/ 32°09'N,77°02'E/ 3,647	64	8x	Abnormal
P-18	Sojha/31°42'N, 77°54'E/ 2,692	32	4x	Normal
P-19	Jalori Pass/ 31°32'N, 77°22'E/ 3,134	64	8x	Normal
P-20	Kullu II/ 32°08'N, 77°04'E/ 2,734	32	4x	Abnormal
P-21	Parvati valley/32°01'N, 77°20'E/1,640	32	4x	Normal
P-22	Manikaran /32°08'N, 77°26'E/ 1,820	32	4x	Normal

intraspecific level revealed significant variations for some of the qualitative characters as is evident from increased stomatal size, guard, and subsidiary cells, stomatal frequency and index in polyploids as compared to diploids (Table 2). Along with these characters, some significant differences were noticed for leaf size in all the three cytotypes (Fig. 1). The size of pollen grain in the octaploid was also found to be significantly larger than their diploid equivalent ($p < 0.05$, Table 2). Such comparable results have been previously reported for many angiosperms such as in *Rorippa amphibia* Besser, 1822 (Luttikhuisen et al. 2007), *Nicotiana glauca* Link & Otto, 1840 (El-Morsy et al. 2009), *Ocimum basilicum* Linnaeus, 1753 (Omidbaigi et al. 2010), etc. These morphological variations may be attributed to the variation in chromosome number as has been reported earlier in *Centaurea stoebe* Ledebur, 1833 (Nakagawa 2006 and Španiel et al. 2008), *Chamerion angustifolium* (Linnaeus, 1753), *Heuchera grossulariifolia* Rydberg, 1900, *Vaccinium corymbosum* Linnaeus, 1753 (Soltis et al. 2007), *Ranunculus parnassifolius* Linnaeus, 1753 (Cires et al. 2009), *Ranunculus hirtellus* Royle, 1753 (Kumar 2011), etc. Overall, increase in ploidy level is correlated with gigantism for some of the vegetative and floral characters.

B) Cytological observations

Based on $x=8$ (Darlington and Wylie 1955), the 22 different populations of *V. jatamansi* examined for cytological variations revealed the existence of diploid ($2n=16$), tetraploid ($2n=32$) and octaploid ($2n=64$) cytotypes. Out of 22 populations, two diploid ($2n=16$) populations showing 8:8 distribution of chromosomes at anaphase-I (Fig. 2a, b) are chromosomally reported for the first time from India. However, diploid ($2n=16$) cytotypes have been previously reported from Germany and Pakistan by Engel (1974), Khatoon and Ali (1993), respectively. The occurrence of eighteen tetraploid cytotypes with 16:16 distribution of chromosomes at anaphase-I (Fig. 2c, d) and is in conformity with the earlier reports from Kashmir Himalayas (Jee et al. 1983). Two other populations with $2n=64$ (32: 32 distribution of chromosomes at anaphase-I) (Fig. 2e) have been cytologically worked out for the first time on worldwide basis.

A perusal of cytological literature reveals that majority of the species in the genus have been worked out showing $2n=14, 16, 28, 32, 42, 48, 56, 60, 64, 72, 80, 90$ and 96 . The genus is dibasic with $x=7, 8$ as has been suggested by Darlington and Wylie (1955). The highest level of ploidy of the genus has been reported to be $12x$ (Engel 1976).

C) Geographical distribution

In the North-Western Himalayas, the distribution pattern of euploid cytotypes shows definite relation to altitudinal variations (Table 1). The distribution of diploids in Himachal Pradesh is uncommon but some accessions are available from lower altitudes of 1350–1900 m. Tetraploids are the most common and are widely distributed within

Table 2. Detailed morphological comparison of three cytotypes of *Valeriana jatamansi*.

No.	Morphological character	Diploid (2n=16)	Tetraploid (2n=32)	Octaploid (2n=64)
1	Distribution	Uncommon	Most Common	Uncommon
2	Habit	Small sized herb	Medium sized herb	Medium sized herb
3	Habitat	Found under shade of <i>Pinus roxburghii</i>	Found in the moist, shady and humus rich places	Found on the slopes in forests
4	Plant height (cm)	14.42±2.84	19.46±2.34	28.70±1.69
Stem				
5	Surface	Non-glabrous	Non-glabrous	Glabrous
6	No. of hairs/ mm ²	-		12.6±1.3
7	Length of hairs (cm)	-	-	2.3±0.38
Basal Leaves				
8	Number of leaves / plant	22.66±7.76	20.77±4.55	47.50±10.60
9	Shape	Ovate	Lanceolate	Lanceolate
10	Size (cm)	4.53±0.95×5.83±1.05	5.56±0.86×6.98±1.01	7.65±0.63×7.26±1.36
11	Surface	Non-glabrous	Non-glabrous	Glabrous
12	Leaves Margin	Toothed	Entire	Wavy
Cauline Leaves				
13	Number of leaves / plant	4.00±1.00	11.41±2.18	21±2.41
14	Size (cm)	4.23±0.70×4.63±0.64	6.53±0.59×7.40±0.74	7.41±2.18×21.00±1.41
15	Surface	Non-glabrous	Non-glabrous	Glabrous
16	Leaves Margin	Toothed	Entire	Entire
Stomata				
17	Size (µm)	21.66±1.56×18.3±0.36	23.82±0.76×19.58±0.67	26.74±0.35×20.55±0.48
18	Stomatal frequency on upper/ lower surface of leaf (mm ²)	5.41±0.45/3.25±0.36	5.48±0.48/7.45±0.45	3.25±0.36/3.78±0.11
19	Stomatal index of upper/ lower surface of leaf (µm)	22.28/12.45	23.45/13.24	24.50/14.32
Inflorescence				
20	Length (cm)	9.9±1.8	10.2±3.5	10.7±1.8
21	Diameter (cm)	2.69±0.23	2.39±0.28	2.34±0.30
22	Number of flowers/plant	9.5±1.58	11±2.16	12.2±1.0
Flower				
23	Petal size (mm)	4.77±0.59	4.06±0.8	4.15±0.91
24	Sepal size (mm)	3.01±0.39	2.99±0.40	3.38±0.5
Pollen size				
25	Pollen size (µm)	37.24±1.15×39.06±0.79	41.05 ± 0.52×44.55 ± 0.59	45.24±0.57×46.19±0.38

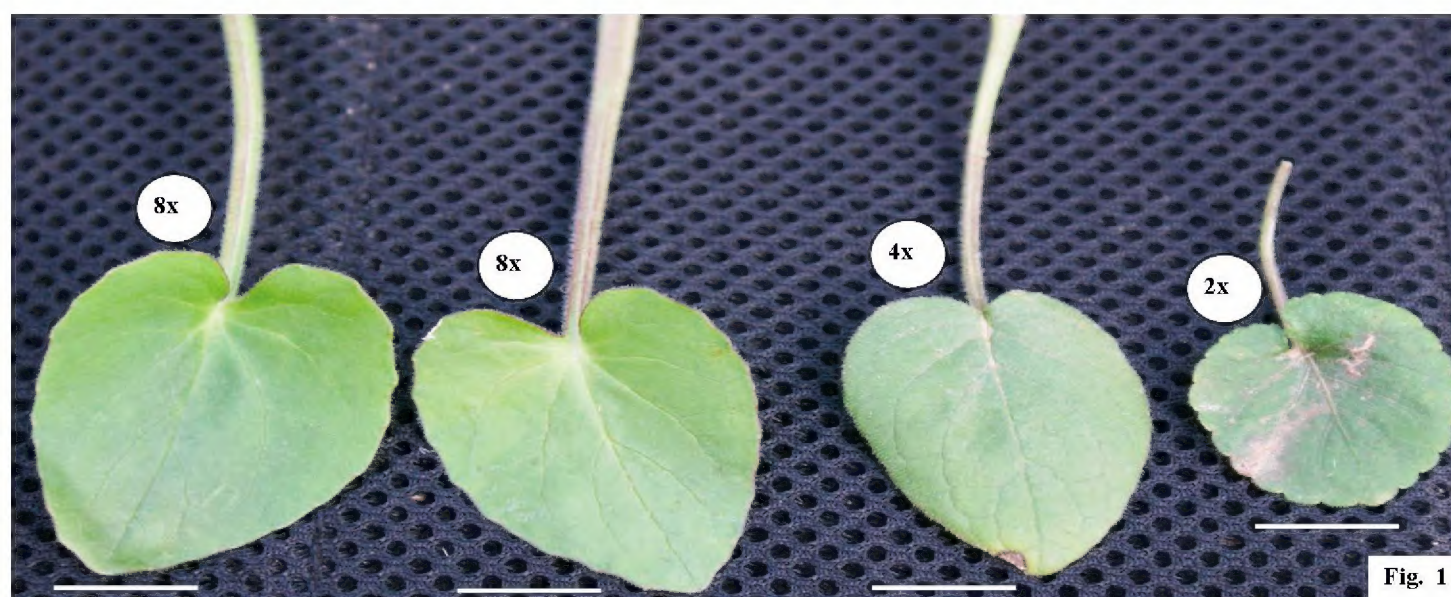


Figure 1. Leaves showing variations among 2x, 4x and 8x cytotypes of *Valeriana jatamansi*.

the altitude of 764–2236 m in the state. The octaploid cytotypes are restricted only to higher altitude localities (3,164–3,647 m) in Kullu district of Himachal Pradesh. Thus it is clear that north-western Himalayan region harbours maximum genetic diversity for the species (Fig. 3).

Meiotic abnormalities

The meiotic course was found to be abnormal in eight populations (Table 3). In such populations, abnormalities in the form of cytomixis, unoriented bivalents, chromatin stickiness, chromatin bridges and laggards, or multipolarity have been observed at different stages of meiosis (Fig. 2f–s; Table 3). Thus in the presently studied populations indicate the existence of intraspecific genetic diversities in the species. Such genetic differences have earlier been reported in different plant species (Sheidai et al. 2003, Rani et al. 2013, Jeelani et al. 2013). The phenomenon of chromatin transfer from early prophase to the pollen formation stage (Fig. 2f–h) has been observed in most of these populations with the highest percentage recorded in populations P-20 from Kullu II (Table 3).

Cytomixis in these populations resulted in the production of hyper- and hypoploid PMCs. According to Sarvella (1958), cytomixis results in some genetic consequences and it is a mechanism to explain the origin of aneuploid gametes. Some others considered it to be of great importance as the most probable consequence of cytomixis is the formation of hypo-, hyperploid and enucleated PMCs, aberrant microspore tetrads and pollen sterility (Sheidai and Fadaei 2005, Jeelani et al. 2011). Cytomixis results in the production of unreduced gametes in several angiosperms and leads to the production of aneuploid or polyploids plants (Fadaei et al. 2010, Mandal et al. 2013). The formation of unreduced gametes is of evolutionary significance in a way that it can lead to the production of plants with higher ploidy level through polyploidization (Villeux 1985). Chromatin stickiness involving few bivalents or whole complement

Table 3. Data on cytomixis, abnormal meiotic course and pollen fertility in *Valeriana jatamansi*.

	Cytomixis at Meiosis-I/ Meiosis II		Meiotic course showing PMCs with				Pollen grains
Population number	% of PMCs involved	Number of PMCs Involved	Chromosomal stickiness at M-I (%)	Bridges at Meiosis-I/ Meiosis-II (%)	Laggards at Meiosis-I/ Meiosis-II (%)	Micronuclei at T-II (%)	Fertility (%)
PP-1	8.33 (10/120)/	2-3	4.00 (4/100)	5.88 (6/102)/	2.86 (2/70)/	17.47 (18/103)	66.23
	7.07 (7/99)			-	2.63 (2/76)		
PP-3	5.78 (7/121)/	2-3	-	3.80 (4/105)/	2.70 (2/74)/	18.18 (16/88)	65.07
				-	2.66 (2/75)		
PP-6	4.34 (5/115)/	2-3	-	2.40 (3/125)/	3.30 (4/121)/	5.78 (7/121)	75.78
	4.59 (4/87)			1.73 (2/115)	-		
PP-10	-	-	4.87 (6/123)	2.27 (3/132)/	-	7.57 (10/132)	70.32
				2.60 (3/115)			
PP-14	5.88 (6/102)/	2-4	9.75 (12/123)	5.35 (6/112)/	5.69 (7/123)/	4.46 (5/112)	71.65
	6.25 (7/112)			3.92 (4/102)	4.83 (6/124)		
PP-16	-	-	7.33 (8/109)	-	3.53 (4/113)/	-	77.87
					-		
PP-17	-	-	8.33 (11/132)	2.52 (3/119)/	-	5.60(7/125)	79.67
				-			
PP-20	9.00 (9/100)/	2-4	-	-	5.73 (7/122)/	4.50(5/111)	79.65
	8.00 (10/125)				-		

was seen from prophase-I to metaphase-I (Fig. 2i–j). Cytomixis and chromatin stickiness are considered to be the result of genetic factors (Bellucci et al. 2003, Ghaffari 2006, Fadaei et al. 2010) and environmental factors (Nirmala and Rao 1996) as well as genomic–environmental interaction (Baptista-Giacomelli et al. 2000) and seems to be equally applicable to the presently investigated populations. Chromatin stickiness also results in the formation of fragmented chromatin (Kumar and Singhal 2011) and at present very low percentage of such fragments was observed.

During present investigations, single or multiple bridges (Fig. 2k) have been recorded in six populations with highest percentage in Population -14 from Prashar. According to Rothfels and Mason (1975) bridges may originate from chiasma formation in heterozygous inversions. Bridges and fragments are the results of spontaneous breakage and fusion of the chromosomes. Early disjunction of bivalents normally does

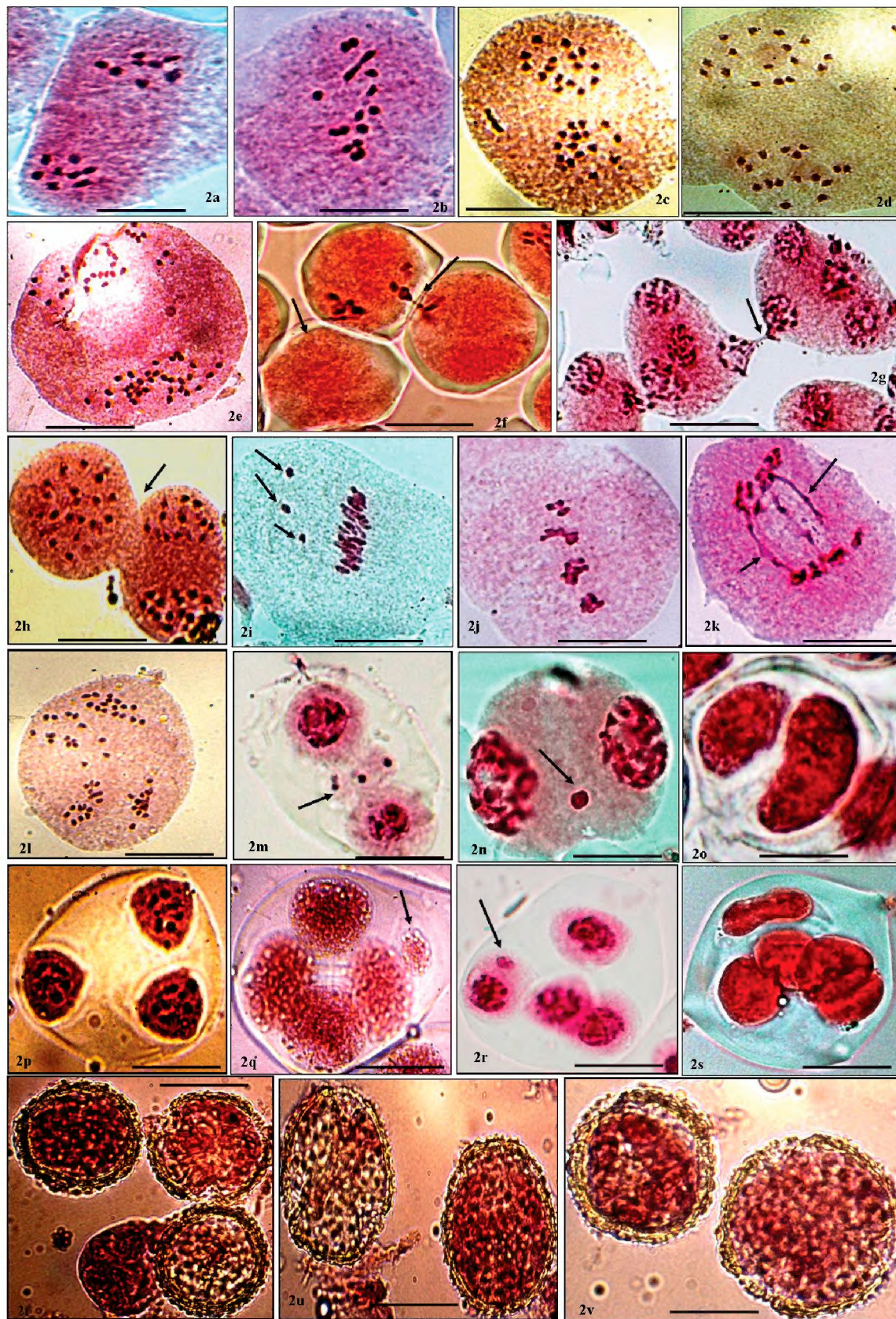


Figure 2. **a** (Diploid cytotype) PMC at Anaphase-I showing 8:8 distribution of chromosomes **b** (Diploid cytotype) PMC at Metaphase-I showing 8II bivalents **c** (Tetraploid cytotype) PMC at Anaphase-I showing 16:16 distribution of chromosomes **d** PMC at Metaphase-II showing 16:16 distribution of chromosome **e** (Octaploid cytotype) PMC at Anaphase-I showing 32:32 distribution of chromosomes **f** (Diploid cytotype) Three PMCs showing transfer of chromatin material (arrowed) **g** A group of PMCs (arrowed) involved in cytomixis at telophase-I **h** (Tetraploid cytotype) Size difference and direct connection between two PMCs during chromatin material (arrowed) **i** PMC at metaphase-I showing unoriented bivalent (arrowed) **j** PMC at metaphase-I showing chromatin stickiness **k** PMC at anaphase-I showing chromatin bridges (arrowed) **l** PMC at anaphase-II showing unequal distribution of chromosomes (arrowed) **m–n** PMC at telophase-I showing chromatin chromosomal laggards (arrowed) **o** Diad **p** Triad **q–r** Tetrad with micronuclei (arrowed) **s** Polyad **t–v** Fertile, sterile and Heterogeneous sized fertile pollen grains. Bar = 10 μ m.

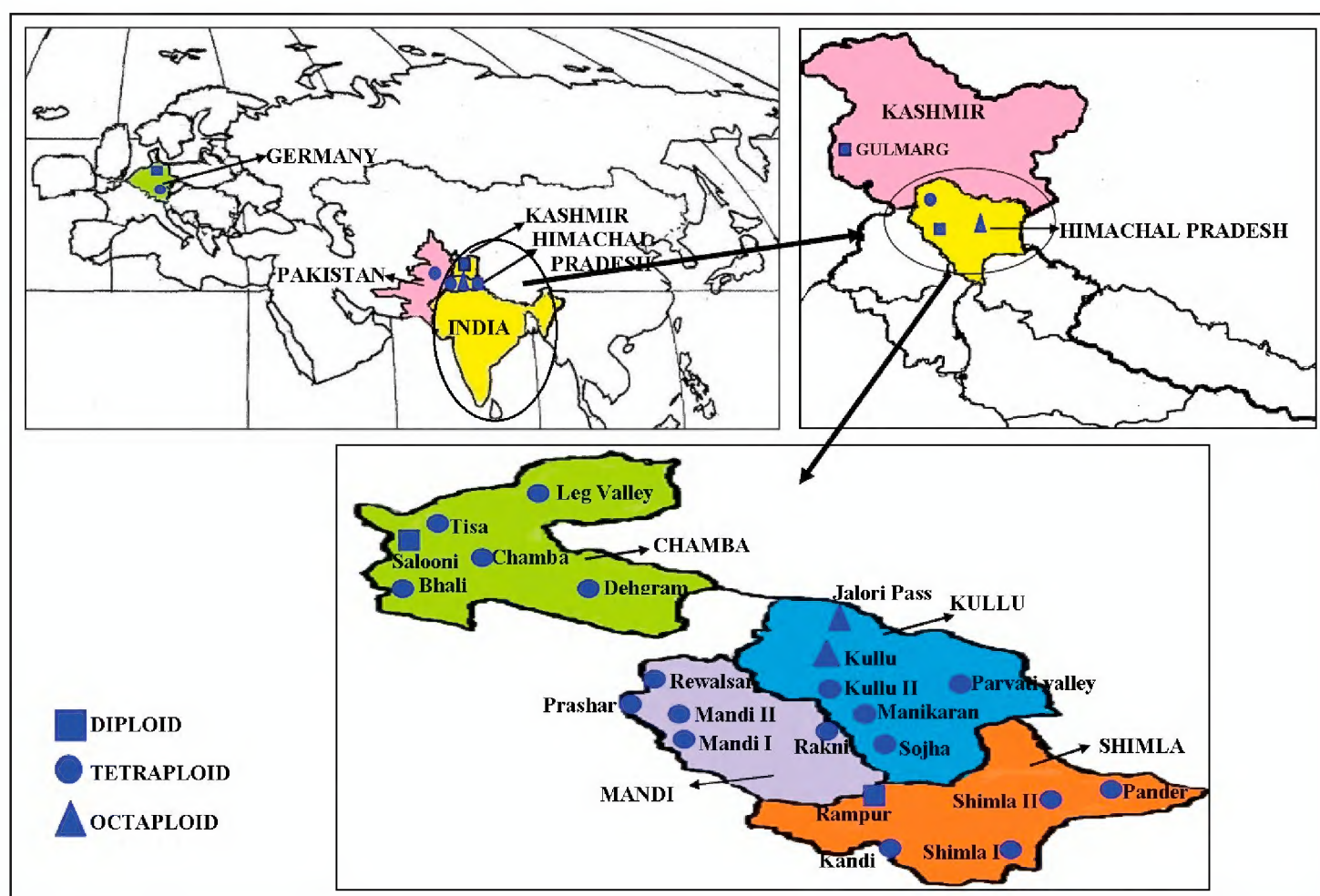


Figure 3. Geographical distribution of 2x, 4x and 8x cytotypes of *Valeriana jatamansi*.

not affect the normal distribution of chromosomes at anaphase-I, but late separation of bivalents which normally exists in hybrids and cytologically abnormal diploids causes some meiotic disturbances (chromatin bridges and laggards) and consequently pollen malformation (Wang et al. 2004). Bivalents and chromosomes that lag behind and are unable to reach at poles during anaphase-I, telophase-I, anaphase- II and telophase-II stages of meiosis form laggards. In our study, chromosomal laggards were noticed in six populations (Fig. 2l–n). There are different explanations for the formation of chromosomal laggards such as interlocking of bivalents and paracentric inversions (Sinha and Godward 1972, Tarar and Dyansagar 1980). One of the most acceptable reasons for the formation of chromosomal laggards is lack of synapsis at early prophase stages or precocious separation and delayed terminalization of chiasmata (Pagliarini 1990, Kumar and Tripathi 2007). All these meiotic abnormalities result in abnormal microsporogenesis, leading to the formation of monads, dyads, triads, or polyads (Fig. 2o–s; Table 4). Furthermore, micronuclei have also been observed in most of these species (Fig. 2q–r; Table 4). These meiotic abnormalities along with abnormal microsporogenesis lead to the formation of heterogeneous sized (large and small) fertile pollen grains and reduced pollen fertility (Fig. 2t–v). The frequency of large-sized pollen grains ranges from 3% to 4% in different populations. The occurrences of large pollen grains conforms to previous information about possibility of such pollen grains to be resulting from unreduced 2n gametes as has been seen in several angiosperms (Bertagnolle and Thomson 1995, Sheidai et al. 2008, Fadaei et al. 2010, Jeelani et al. 2011). Presence of genetic diversity within the populations of *V. jatamansi* stresses the need for further cytological analysis

Table 4. Data on abnormal microsporogenesis in different cytotypes of *Valeriana jatamansi* marked with abnormal meiosis.

Microsporogenesis Accessions								
	Dyads		Triads		Tetrads		Polyads	
	WMN	WN	WMN	WN	WMN	WN	WMN	WN
PP-1	0.99 (1/101)		0.99 (1/101)	1.98 (2/101)	71.28 (72/101)	21.78 (22/101)	1.98 (2/101)	-
PP-3	-	-	2.04 (2/98)	-	76.53 (75/98)	21.42 (21/98)	-	-
PP-6	0.96 (1/104)	-	0.96 (1/104)	-	70.19 (73/104)	26.92 (28/104)	0.96 (1/104)	-
PP-10	2.04 (2/98)	1.02 (1/98)	2.04 (2/98)	-	88.77 (87/98)	6.12 (6/98)	-	-
PP-14	2.97 (3/101)	0.99 (1/101)	3.96 (4/101)	0.99 (1/101)	79.20 (80/101)	9.90 (10/101)	0.99 (1/101)	0.99 (1/101)
PP-16	-	0.94 (1/106)	2.83 (3/106)	0.94 (1/106)	84.90 (90/106)	10.37 (11/106)	-	-
PP-17	-	-	0.98 (1/102)	0.98 (1/102)	89.21 (91/102)	8.82 (9/102)	-	-
PP-20	1.86 (2/107)	0.93 (1/107)	3.73 (4/107)	0.93 (1/107)	76.63 (82/107)	14.01 (15/107)	0.93 (1/107)	0.93 (1/107)

WMN = without micronuclei; WM = with micronuclei.

from different geographical areas. Intraspecific variability at population level has been brought to the fore, to be conserved and/or utilized for further plant improvement programme. The genetic diversity in *V. jatamansi* points towards the need of studies for chemical and molecular characterization and biological activity of these variants to identify superiour chemotypes for further conservation and exploitation.

Acknowledgements

The authors are grateful to the Science and Engineering Research Board (SERB) under young Scientist Fellowship Scheme [Registration No. SERB/LS-527/2013] to Dr. Savita Rani. Thanks are also due to the Head, Department of Agricultural Biotechnology, CSK, Himachal Pradesh Agricultural University, Palampur, for necessary laboratory facilities.

References

Bagchi P, Hopper W (2011) Virtual Screening of Active Compounds of *Valeriana wallichii*, *Acorus calamus* and *Asparagus racemosus* with Schizophrenic Proteins COMT & GRM3. International Proceedings of Chemical, Biological and Environmental Engineering 5: 15–22.

Baptista-Giacomelli FR, Palgliarini MS, Almeida JL (2000) Meiotic behavior in several Brazilian oat cultivars (*Avena sativa* L.). Cytologia 65: 371–378. doi: 10.1508/cytologia.65.371

- Bell CD (2004) Preliminary phylogeny of Valerianaceae (Dipsacales) inferred from nuclear and chloroplast DNA sequences data. *Molecular Phylogenetics and Evolution* 31: 340–350. doi: 10.1016/j.ympev.2003.07.006
- Belling J (1921) On counting chromosomes in pollen mother cells. *The American Naturalist* 55: 573–574. doi: 10.1086/279843
- Bellucci M, Roscini C, Mariani A (2003) Cytomixis in pollen mother cells of *Medicago sativa* L. *Journal of Heredity* 94: 512–516. doi: 10.1093/jhered/esg096
- Bennet SSK (1987) Name changes in flowering plants of India and adjacent regions. Dehradun, 583 pp.
- Bertagnolle F, Thomson JD (1995) Gametes with the somatic chromosome number: mechanisms of their formation and role in the evolution of autopolyploid plants. *New Phytologist* 129: 1–22. doi: 10.1111/j.1469-8137.1995.tb03005.x
- Cires E, Cuesta C, Peredo EL, Revilla MA, Fernández Prieto JA (2009) Genome size variation and morphological differentiation within *Ranunculus parnassifolius* group (Ranunculaceae) from calcareous screes in the Northwest of Spain. *Plant Systematics and Evolution* 281: 193–208. doi: 10.1007/s00606-009-0201-9
- Chen HB, Zhu JM, Zhao YY, et al. (1999) Chemotaxonomical studies on *Valeriana officinalis* Linn. and its relative species from China. *Journal of Traditional Chinese Medicine* 21: 652–654.
- Chopra RN, Nayyar SO, Chopra IC (1956) Glossary of Indian Medicinal Plants. CSIR Publication, New Delhi, 251 pp.
- Darlington CD, Wylie AP (1955) Chromosome Atlas of Flowering Plants. London, 519 pp.
- El-Morsy ShI, Dorra MDM, Elham AAAEl-H, Atef AAH, Ahmed YM (2009) Comparative studies on diploid and tetraploid levels of *Nicotiana glauca*. *Academic Journal of Plant Sciences* 2: 182–188.
- Engel K (1976) Birtrage zur Systematik der Valerianaceae unter besonderes Berücksichtigung cytosystematischer Ergebnisse. Dissertation, Giessen, 196 pp.
- Fadaie F, Sheidai M, Asadi M (2010) Cytological study the genus *Arenaria* L. (Caryophyllaceae). *Caryologia* 63: 149–156. doi: 10.1080/00087114.2010.589720
- Fedorov AA (1969) Chromosome Numbers of Flowering Plants. Academy of Sciences of the USSR. Komarov Botanical Institute, Leningard (Reprint 1974), 550 pp.
- Fernald ML (1950) Gray's Manual of Botany. A handbook of the flowering plants and ferns of the central and northeastern United States and Canada. New York, 1632 pp.
- Ghaffari SM (2006) Occurrence of diploid and polyploid microspores in *Sorghum bicolor* (Poaceae) is the result of cytomixis. *African Journal of Biotechnology* 5: 1450–1453.
- Hodálová I, Crulich V, Horová L, Valachovic M, Marhold K (2007) Occurrence of tetraploid and octoploid cytotypes in *Senecio jacobaea* ssp. *Jacobaea* (Asteraceae) in Pannonia and the Carpathians. *Botanical Journal of the Linnean Society* 153: 231–242. doi: 10.1111/j.1095-8339.2007.00605.x
- Jee V, Dhar U, Kachroo P (1983) In IOPB chromosome number reports LXXIX. *Taxon* 32: 321.
- Jeelani SM, Rani S, Kumar S, Kumari S, Gupta RC (2011) Meiotic studies in some members of Caryophyllaceae Juss. from the Western Himalayas. *Acta Biologica Cracoviensia. Series Botanica* 53: 86–95. doi: 10.2478/v10182-011-0012-5

- Jeelani SM, Kumari S, Gupta RC, Siddique MAA (2013) Detailed cytomorphological investigations through male meiosis of polypetalous plants from the Kashmir Himalaya. *Plant Systematics and Evolution* 300: 1175–1198. doi: 10.1007/s00606-013-0954-z
- Khatoon S, Ali SI (1993) Chromosome Atlas of the Angiosperms of Pakistan. Department of Botany, University of Karachi, Karachi, 220 pp.
- Kour R, Sood M, Chander M, Mahajan R, Kumar V, Sharma DR (1999) Plant Cell Tissue and Organ Culture 59: 227–229. doi: 10.1023/A:1006425230046
- Kumar P (2011) Exploration of cytomorphological diversity in the members of Polypetalae from Lahaul-Spiti and adjacent areas. PhD Thesis, Punjabi University Patiala, India, 204–213. doi: 10.1007/s00606-011-0516-1
- Kumar P, Singhal VK (2011) Chromosome number, male meiosis and pollen fertility in the plants of Polypetalae from Indian cold deserts of Lahaul- Spiti and adjoining areas in Himachal Pradesh (India). *Plant Systematics and Evolution* 297: 271–297.
- Kumar V, Subramaniam B (1986) Chromosome Atlas of Flowering Plants of the Indian Sub-continent. Vol. I. Dicotyledons. Botanical Survey of India, Calcutta.
- Kumar G, Tripathi R (2007) Anomalous nucleolar and chromosomal organization in induced phenodeviants of grass pea. *Cytologia* 79: 345–350. doi: 10.1508/cytologia.72.345
- Kumar S, Jeelani SM, Rani S, Gupta RC, Kumari S (2013) Cytology of five species of subfamily Papaveroideae from the Western Himalayas. *Protoplasma* 250: 307–316. doi: 10.1007/s00709-012-0413-7
- Lihová J, Tribsch A, Marhold K (2003) The *Cardamine pratensis* (Brassicaceae) group in the Iberian Peninsula: taxonomy, polyploidy and distribution. *Taxon* 52: 783–802. doi: 10.2307/3647352
- Luttikhuisen PC, Stift M, Kuperus P, Van Tienderen PH (2007) Genetic diversity in diploid vs. tetraploid *Rorippa amphibia* (Brassicaceae). *Molecular Ecology* 16: 3544–3553. doi: 10.1111/j.1365-294X.2007.03411.x
- Mandal A, Datta AK, Gupta S, Paul R, Saha A, Ghosh BK, Bhattacharya A, Iqbal M (2013) Cytomixis-a unique phenomenon in animal and plant. *Protoplasma* 250: 985–996. doi: 10.1007/s00709-013-0493-z
- Marks GE (1954) An acetocarmine glycerol jelly for use in pollen fertility counts. *Stain technology* 29: 277.
- Moore RJ (Ed.) (1970, 1971, 1972, 1973, 1974, 1977) Index to Plant Chromosome Numbers 1967–1971, 1968, 1969, 1970, 1972, 1973–1974. *Regnum Veg.* 68, 77, 84, 90, 91, 96.
- Nakagawa M (2006) Ploidy, geographical distribution and morphological differentiation of *Parasenecio auriculata* (Senecioneae, Asteraceae) in Japan. *Journal of Plant Research* 119: 51–61. doi: 10.1007/s10265-005-0239-x
- Nirmala A, Rao PN (1996) Genesis of chromosomal numerical mosaicism in higher plants. *Nucleus* 39: 151–175.
- Omidbaigi R, Mirzaee M, Hassani ME, Sedghi Moghadam M (2010) Induction and identification of polyploidy in basil (*Ocimum basilicum* L.) medicinal plant by colchicine treatment. *International Journal of Plant Production* 4(2): 87–98.
- Pagliarini MS (1990) Meiotic behavior and pollen fertility in *Aptenia cordifolia* (Aizoaceae). *Caryologia* 43: 157–162. doi: 10.1080/00087114.1990.10796994

- Prakash V (1999) Indian Valerianaceae - A Monograph on Medicinally Important Family, vol. 1–2. Scientific Publishers, India.
- Rani S, Kumari S, Gupta RC, Chahota RK (2015) Cytological studies of Angiosperms (174 species) from District Kangra, Himachal Pradesh (India). Plant Systematics and Evaluation 300: 851–862. doi: 10.1007/s00606-013-0924-5
- Rothfelsk H, Mason GF (1975) Achiasmate meiosis and centromere shift in *Eusimulium aureum* (Diptera: Simuliidae). Chromosoma 51: 111–124. doi: 10.1007/BF00319829
- Sarvella P (1958) Cytomixis and loss of chromosomes in meiotic and somatic cells of *Gossypium*. Cytologia 23: 14–24. doi: 10.1508/cytologia.23.14
- Sheidai M, Fadael F (2005) Cytogenetic studies in some *Bromus* L. species. Journal of Genetics 84: 189–194.
- Sheidai M, Koobaz P, Zehzad B (2003) Meiotic studies of some *Avena* species and populations in Iran. Journal of Sciences, Islamic Republic of Iran 14: 121–131.
- Sheidai M, Nikoo M, Gholipour A (2008) Cytogenetic variability and new chromosome number reports in *Silene* L. species (Sect. Lasiostemones, Caryophyllaceae). Acta Biologica Szegediensis 52: 313–319.
- Sinha SSN, Godward MBE (1972) Radiation studies in *Lotus* cultivars. Meiosis: abnormalities due to gamma radiation and its consequences. Cytologia 37: 685–698. doi: 10.1508/cytologia.37.685
- Soltis DE, Soltis PS, Schemske DW, Hancock JF, Thompson JN, Husband BC, Judd WS (2007) Autopolyploidy in Angiosperms: have we grossly underestimated the number of species? Taxon 56: 13–30.
- Španiel S, Marhold K, Hodálová I, Lihová J (2008) Diploid and tetraploid cytotypes of *Centaurea stoebe* (Asteraceae) in central Europe: morphological differentiation and cytotype distribution patterns. Folia Geobotanica 43: 131–158. doi: 10.1007/s12224-008-9008-7
- Svensson S (1983) Chromosome numbers and morphology in the *Capsella bursa-pastoris* complex (Brassicaceae) in Greece. Willdenowia 13(2): 267–276.
- Tarar JL, Dyansagar VR (1980) Effect of gamma rays and EMS on growth and branching in *Turnera ulmifolia* L. Journal Cytology and genetics 14: 118–124.
- Villeux R (1985) Diploid and polyploidy gametes in Crop Plants: Mechanisms of formation and utilization in plant breeding. In: Janick J (Ed.) Plant Breed Rev 3. AVI Publishing Co. Wesport, Connecticut, 442.
- Wang SY, Yui CK, Lee S, Wang CI, Chzan GC (2004) Ultrastructure and possible origin of the cytoplasmic channels providing connection between cells of anther vegetative tissues. Russian Journal of Plant Physiology: A Comprehensive Russian Journal on Modern Phytophysiology 51: 110–120.